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INCREASED RATE OF METABOLISM OF EPINEPHRINE AND NOREPINEPHRINE BY SYMPATHOMIMETIC AMINES

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It has been previously shown that many types of drugs alter the rate of metabolism of the catecholamine hormones (Axelrod and Laroche, 1959; Axelrod and Tomchick, 1959; Axelrod, 1960). The psychotropic drugs reserpine, chlorpromazine and imipramine speed the metabolism of epinephrine and norepinephrine, while the catechol-O-methyl transferase inhibitors, pyrogallol and quercetin, block the metabolism of these compounds. Although iproniazid, a monoamine oxidase inhibitor, does not affect the rate of disappearance of catecholamines themselves, it blocks the deamination of their O-methylated metabolites, metanephrine and normetanephrine (Axelrod *et al.*, 1958). In this communication we wish to report that a number of sympathomimetic amines increase the rate of disappearance of epinephrine and norepinephrine in the intact mouse.

MATERIALS AND METHODS. Adult male mice (NIH stock) weighing 25 to 30 g were used. H^3-Epinephrine bitartrate (267 μc/mg) and H^3-7-norepinephrine bitartrate (1720 μc/mg) were prepared by the New England Nuclear Corp., Boston, Mass. These compounds were chromatographically pure and when dissolved in 2% sodium metabisulfite and kept at −10°C did not decompose or lose tritium after several months' storage.

Estimation of H^3-epinephrine and H^3-norepinephrine in whole mouse. Mice were killed by a blow on the head and the whole animal was homogenized with 100 ml of 0.1 N HCl in a Waring blendor. An aliquot of the homogenate was centrifuged at 10,000 × *g* and H^3-epinephrine was determined as described previously (Axelrod *et al.*, 1959b). H^3-Norepinephrine was determined by the same procedure, except that the catecholamine was eluted from the alumina column with 0.1 N HCl.

Specificity of the method. Eluates from whole mouse extracts obtained by the procedure described above were applied to Whatman #1 paper and developed in butanol–acetic acid–water, 8:2:2, or in phenol saturated with water and SO_2. Depending on the catecholamine administered, single peaks of radioactivity having the same R_f's as authentic epinephrine or norepinephrine were found.

Catechol-O-methyl transferase activity was determined, using H^3-epinephrine as a substrate, by a procedure described previously (Axelrod *et al.*, 1959a).

RESULTS. *Disappearance of epinephrine and norepinephrine in the whole mouse.* Mice were given 3 μg H^3-epinephrine (free base) or 1 μg norepinephrine (free base) in the tail vein. Ten minutes later the mice were killed and assayed for epinephrine or norepinephrine remaining. Zero time values were obtained by homogenizing untreated mice and then adding 3 μg epinephrine or 1 μg norepinephrine to the whole homogenate. After 10 minutes about 38% of the administered epinephrine was found in the mouse (table 1). The rate of metabolism of the hormone in the mouse was the same after the administration of 1 to 10 μg epinephrine. The disappearance of norepinephrine was slower than that of epinephrine, about 56% remaining after 10 minutes.

Effect of sympathomimetic amines on the metabolism of catecholamines in vivo. The rate of metabolism of epinephrine and norepinephrine in the whole mouse after the administration of various sympathomimetic amines was examined. Mice were first given the sympathomimetic amine and then the catecholamine was administered intravenously. Details of drug dosage are described in table 1. The animals were killed 10 minutes after the injection of the catecholamine and assayed for the amount of hormone remaining. The sympathomimetic amines phenylethylamine, tyramine, phenylaminobutane, *d*-amphetamine and ephedrine markedly increased the rate of metabolism of catecholamines in the whole mouse; Paredrine (*p*-hydroxyamphetamine) had a moderate effect, and Synephrine

TABLE 1

Augmentation of catecholamine metabolism by sympathomimetic amines

Drug	Amount of Drug Given Each Mouse*	Time Drugs Were Given before Catecholamine	Catecholamine Remaining in Whole Mouse	
			Epinephrine	Norepinephrine
	mg/kg	*min*	%	%
None			38 ± 4.5	56 ± 5.1
Phenylethylamine	100	10	18 ± 3.4	15 ± 3.1
Ephedrine	50	10	20 ± 3.1	22 ± 2.2
d-Amphetamine	20	10	18 ± 3.5	19 ± 2.6
Tyramine	80	30, 10	22 ± 3.3	35 ± 4.0
Phenylaminobutane	40	10	23 ± 4.5	37 ± 2.5
Paredrine	40	10	27 ± 2.2	34 ± 5.3
Synephrine	40	10	35 ± 8.8	54 ± 3.5

* All sympathomimetic amines were given intraperitoneally. Mice received 3 μg H^3-epinephrine or 1 μg norepinephrine in the tail vein and were killed 10 minutes later. Results are expressed as per cent of administered catecholamine remaining in the whole animal (± standard deviation of the mean) in 10 minutes. Six to 10 mice were used in each group.

(*p*-hydroxy(methylaminomethyl) benzyl alcohol) had a negligible effect.

Lack of effect of sympathomimetic amines on catechol-O-methyl transferase. Almost all of the epinephrine and norepinephrine that disappeared in the whole mouse could be accounted for as free and conjugated O-methylated metabolites metanephrine (Axelrod and Laroche, 1959) and normetanephrine (Whitby *et al.*, 1960). Since the catecholamines were metabolized primarily by O-methylation, the sympathomimetic amines might elicit their response by enhancing catechol-O-methyl transferase activity. Consequently, the effect of sympathomimetic amines on catechol-O-methyl transferase activity was examined. The enzyme was prepared by homogenizing the mouse liver in 5 volumes of ice-cold isotonic potassium chloride in a Waring blendor. After centrifuging the homogenate at 4°C at 80,000 × *g* for 30 minutes, an aliquot of the clear soluble supernatant fraction was assayed for catechol-O-methyl transferase activity (Axelrod *et al.*, 1959a) (at a concentration of 5×10^{-4} M and 5×10^{-5} M). Sympathomimetic amines (*d*-amphetamine, ephedrine, tyramine, phenylethylamine) had no effect on catechol-O-methyl transferase activity.

Discussion. We have shown that epinephrine (Axelrod *et al.*, 1959b) and norepinephrine (Whitby *et al.*, 1960) disappear from the whole animal in two phases. In the first 5 minutes, part of the catecholamines are rapidly metabolized, primarily by O-methylation. Following this, the catecholamines disappear slowly over a period of many hours. The slower second phase indicates that part of the catecholamines are bound to tissue constituents and then slowly released and metabolized. Since a larger fraction of norepinephrine is found in the whole mouse as compared to epinephrine, it appears likely that a larger amount of norepinephrine is bound. Reserpine, a compound that has been shown to release catecholamines from tissue (Holzbauer and Vogt, 1956), also speeds the metabolism of epinephrine (Axelrod and Tomchick, 1959) and norepinephrine (Axelrod, 1960). This compound is presumably acting by preventing the binding of the administered catecholamines, thus exposing them to enzymatic attack and more rapid destruction. The findings reported here, that sympathomimetic amines increase the rate of metabolism of epinephrine and norepinephrine, suggest that they might also prevent the protective binding by tissue constituents.

Many hypotheses have been proposed explaining the mode of action of sympathomimetic amines. Until recently, it was generally believed that these compounds act directly on the adrenergic receptors or by inhibiting monoamine oxidase. Carlsson *et al.* (1957) and Burn and Rand (1958) have shown that sympathomimetic amines will not elicit their responses in the absence of tissue catecholamines. As a result of these observations, Burn and Rand (1958) postulated that sympathomimetic amines act by liberating catecholamines. Our findings tend to

support such a mechanism. We have shown that sympathomimetic amines speed the metabolism of administered catecholamines, presumably by preventing the protective binding of these hormones. By affecting the binding sites, the sympathomimetic amines might also cause the release of bound endogenous catecholamines. Burn and Rand (1958) have found that tyramine, phenylethylamine, ephedrine and *d*-amphetamine will not produce a pressor action when the catecholamine stores are depleted. Likewise, we have noted that these compounds also increase the rate of metabolism of catecholamines. On the other hand, it has also been observed by Burn and Rand (1958) that Neosynephrine (phenylephrine) increased pressor response in animals depleted of catecholamines. Synephrine, a congener of Neosynephrine, had little effect on the speed of metabolism of catecholamines.

SUMMARY

Epinephrine disappears from the whole mouse more rapidly than norepinephrine.

The sympathomimetic amines tyramine, phenylethylamine, ephedrine, *d*-amphetamine, phenylaminobutane and Paredrine (*p*-hydroxyamphetamine) markedly increase the rate of disappearance of epinephrine and norepinephrine in the whole mouse.

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